THE INFLUENCE OF TEST MATERIALS ON REPRODUCTION IN PONTIGULASIA VAS (LEIDY) SCHOUTEDEN

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The test of *Pontigulasia vas* resembles that of *Difflugia* and other closely allied forms. It consists of small sand grains cemented together. These sand grains are collected by the animals before division and are formed into the new shell for one of the daughter individuals. According to Pateff (1926), Gruber in 1886 first described division in *Difflugia*. More recently work on division in these forms has been done by Zuelzer (1904), Goette (1916), Pateff (1926), and others.

While experimenting with *Pontigulasia vas*, it was found that reproduction could be prevented if the cultures were kept free of substances used in shell construction. Division here was apparently related to an inert foreign substance since the animals had plenty of food and were in good condition. It was then decided to investigate this problem further in the hope that the relationship between test materials, mitosis and division in this form could be made more clear.

The writer takes pleasure in expressing his appreciation for the interest of Dr. B. D. Reynolds, under whose direction this work was carried on.

Material and Methods

The *Pontigulasia* used in these experiments were collected from a number of ponds near Charlottesville, Virginia. After isolation and identification the animals were placed in Syracuse watch glasses with clean *Spirogyra*. These watch-glass cultures were kept in moist chambers. The *Pontigulasia* were examined daily, using the wide field binocular microscope, their number noted, and the water in the cultures changed. All water for these cultures was filtered and the pH value taken before using. During the time of the experiments the pH value ranged from 6.3 to 6.9. Where sand or other substances were used these materials were obtained as clean as possible and crushed in a mortar with water. The smaller fragments and water were then transferred to the cultures with pipettes. Observations on the *Pontigulasia* while gathering materials for shell building were made

with the 16 or 8 mm. objective of a microscope. Both photomicrographs and camera lucida drawings were made of these animals. It was found that serial sections of *Pontigulasia* provided the only satisfactory method to show the protoplasmic structures within the shell. Sectioning was made possible by removing the silica dioxide (SiO_2) of the shell particles with hydrofluoric acid (H_2F_2). The *Pontigulasia* were fixed with Schaudinn's or Bouin's fluid and placed in a solution of 70 per cent alcohol and 2 per cent hydrofluoric acid. This solution was kept in paraffin-coated dishes. Ten to fourteen days were needed to remove the silica. Stronger solutions were tried but damaged fixation in most cases. Following removal of the silica, the *Pontigulasia* were imbedded in paraffin by the usual method.

TABLE I

The culturing of *Pontigulasia* without shell materials and the effect on division. Cultures with shell materials are the controls.

Culture number	Material for shell	Number of animals at start	Time	Number of animals at finish	Number of divisions
			days		
1	None	5	17	5	0
Control	Glass	5	17	10	5
2	None	5	21	5	0
Control	Sand	5	21	18	13
3	None	3	33	3	0
Control	Sand	3	33	30	27
4	None	5	26	6	1
Control	Sand	5	10	10*	5

* This was accidentally destroyed on the tenth day.

Sectioning was done at 7 μ. For staining Heidenhain's iron hematoxylin and Delafield's hematoxylin were used.

EXPERIMENTS

The following experiments were made to test the effect of culturing *Pontigulasia vas* without shell materials. The cultures were run in pairs, one was supplied with powdered sand or glass, the other was not. Beside this difference they were as far as possible exactly alike in composition and received the same treatment during the course of the experiments. The cultures with materials for shell building were considered the controls. Table I gives the results of these experiments in condensed form.

The only negative result was in Culture No. 4 where one division apparently took place. Two explanations can be given. This division was noticed the second day after the culture was started and the animal,

which was small, might have been overlooked before. Secondly there might have been enough sand in the *Spirogyra* to furnish materials for the shell. This one negative result can hardly affect the conclusion that *Pontigulasia vas* will not divide unless materials for shell building are present.

It was then thought that perhaps mitotic division had taken place resulting in multinucleate forms similar to those produced in *Amœba proteus* by Chalkley and Daniel (1933). Sections of *Pontigulasia vas* which had been cultured without shell materials showed them to be in a normal condition. There was only one nucleus and, as far as could be determined, the cytoplasm appeared unaffected.

The rest of these *Pontigulasia* were given sand to determine if their power of reproduction had been affected. After some delay division took place. An individual from Culture 3 gave a typical reaction. This animal made no effort at first to collect shell materials but began to do so three days later. By the fourth day it had produced a normal offspring. It appears, therefore, that the power of reproduction had not been permanently affected.

During the experiments the actions of the *Pontigulasia* without shell materials were interesting. Much of the time was spent moving about on the bottom of the watch glasses without any attempt to feed. At such times the pseudopods would become ragged in outline with a wide hyaline area at the ends. This type of pseudopod is usually associated with the collection of test materials. Undoubtedly these animals would have collected sand had it been present. After a day or two of such moving about the animals would begin to feed again. At other times they would go into a quiescent state for several days before feeding.

Some work was done in an attempt to find when nuclear changes began in relation to the sand-gathering activities. A number of *Pontigulasia* were isolated for several days with plenty of food. Finely ground sand was then added and the animals began to collect it. The gathering of shell materials took from 4½ to 5 hours. The pseudopods were next withdrawn and the new shell was formed immediately in front of the mother individual. During this process a number of animals were fixed and sectioned. It was found that mitosis was not begun until the collecting process had been almost completed. Fig. 1, *a*, shows a section of a *Pontigulasia* which had been collecting shell materials for four hours at the time of fixation. Nuclear changes here are very slight. There is, however, a decided mass of chromatin in the center of the nucleus. The sand grains in this form are collected and held outside the mother shell similar to *Difflugia urceolata* as found by

Zuelzer (1904). The vacuoles in the cytoplasm protruding from the shell are pockets from which the sand grains have been dissolved.

Fig. 1, *b*, shows an animal which was fixed just after the collection of shell materials had been completed but before the new shell was formed. The nucleus in this animal is in middle prophase, showing the chromatin granules on a definite linin reticulum. At this stage the nucleus is much enlarged. This section also shows the details of the shell very clearly. The internal diaphragm can be seen with the cytoplasm flowing through it. This diaphragm is easily overlooked in the living condition and the constriction between the neck and the

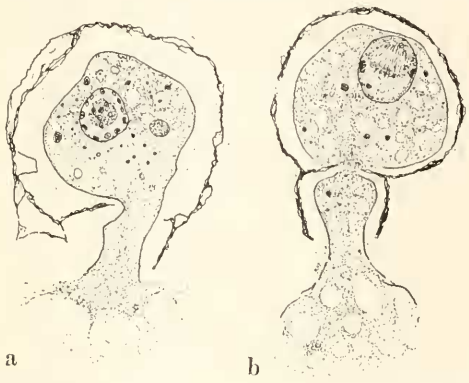

FIG. 1. Sections of *Pontigulasia vas* fixed while gathering test materials before division. Camera lucida drawings. × 400.

a. This animal had been collecting fine sand grains for 4 hours. The shell in this section was distorted when sectioned. Note that the nucleus is scarcely changed from the resting condition.

b. Section of an animal which had been collecting for 4½ hours. The formation of pseudopods had ceased but the sand grains were still a formless mass in front of the mouth of the old shell. The nucleus is much enlarged and shows a definite linin reticulum with the chromatin granules. Middle prophase. Note the constriction and the internal diaphragm in the shell.

fundus is not as deep in these forms as described by Penard (1902), Cash (1909), and Kudo (1931).

The process of division was not investigated further in this study, but it seems fairly certain that nuclear division is not started until the collecting of shell materials has been completed. A more complete study of binary fission in this form is being made.

DISCUSSION

As far as these experiments on the control of division in *Pontigulasia vas* were carried, it seems that reproduction can be prevented by withholding shell materials. It is possible that division might have

taken place if the cultures had been continued over a longer period of time. This appears unlikely, however, for in some uncontrolled cultures animals have been kept as long as 70 days without reproduction.

There still remains the possibility that individuals without shells were produced. If so, these naked animals could not have survived long for no evidences of them were seen.

No definite effect of inhibited division can be given here. There was a tendency for animals in cultures without sand to go into an inactive state immediately after attempting to collect shell materials. This inactive state lasted from one to three days. Also when such individuals were given sand there appeared to be a delay in collecting it and a delay in division afterwards. The animals resulting from such division, however, were normal as far as determined. If these variations are a result of inhibited division then they must be considered in the work done on nuclear division and its relation to shell materials. The animals used in this part of the work were kept without sand for a short time only in an effort to reduce any error from this source.

Nuclear division in *Pontigulasia vas* appears to be dependent on the shell materials in the cytoplasm rather than the collecting action of the animal itself. As the collecting process nears completion, the *Pontigulasia* seem to be in a very unstable physiological condition. If they are disturbed in any way the sand grains will be discarded and division will not take place. Pateff (1926) found the same condition in *Difflugia mammallaris* when about to reproduce.

Summary

1. *Pontigulasia vas* will not reproduce unless shell materials are present in the cultures.

2. The shell materials are collected for a period of approximately four hours immediately before division.

3. There are no changes in the nucleus until almost all the shell materials are collected. Nuclear changes are begun, however, before the formation of the new shell.

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